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FOREWORD

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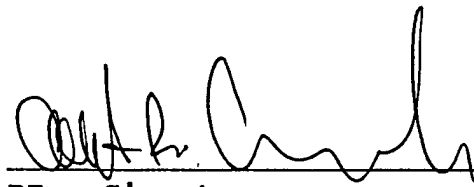
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Introduction

Environmental estrogens have been implicated as etiological agents for breast cancer. This study is aimed at identifying mechanistic information regarding the estrogenicity and carcinogenicity of environmental estrogens through structure-activity relationship (SAR) and quantitative SAR (QSAR) modeling. To facilitate this investigation, the computer-based expert-system MULTICASE is used in addition to other molecular modeling techniques. Learning sets have been derived from bioassays designed to detect estrogenic chemicals. These databases include an *in vitro* estrogen competitive binding assay, an *in vitro* MCF-7 cell proliferation assay and a whole animal uterine weight increase assay (currently being derived). The SAR and QSAR models derived from these databases are considered in conjunction with preexisting SAR and QSAR models for rodent carcinogenicity as well as for other toxicological phenomena to identify features that are common to estrogenicity and carcinogenicity.

Body

The past year has been very exciting because I was awarded my Ph.D. As part of the rigors of writing my dissertation and preparing for the defense, I did not have the necessary time to commit to the completion of this research project. I have been granted a one year extension by the USAMRMC. It must be strongly noted that due to the support through this project, I was able to excel in my academic pursuits. Moreover, research conducted to date as part of this project have laid the groundwork for further research opportunities. Additionally, a position of assistant professor was offered to me from the department from which I graduated. As part of accepting this offer, I have verified that I will have the time and resources for this project's successful completion. I believe that this position was offered to me, in part, due to the University of Pittsburgh's understanding of the importance of breast cancer research as well as my particular area of interest in computational toxicology and its successful applications to the investigation into the etiology of breast disease.

To date, SAR models have been created and published describing mouse [1] and rat [2] carcinogens. These models will serve as the basis for further comparisons for the estrogen SAR models.

Previously in our laboratory, we had a standard method for assessing the structural and mechanistic similarity between various SAR models. A series of comparisons between diverse SAR models is conducted. CASE biophores derived from several SAR models are used. Basically, structural overlap is defined as a biophore from one SAR model being identical to a biophore in another SAR model or being embedded in a biophore from another SAR model. For example, an overlap would be noted in either of the following cases; Cl-CH₂ from model 1 is identical to Cl-CH₂ from model 2, or CH=N- from model 1 is imbedded in CH=N-C=CH- from model 2. For statistical analyses, overlap with α 2 μ -induced nephropathy, inhibitors of cytochrome P450 2D and binding to cytosolic Ah receptor have served as the null hypothesis in that minimal mechanistic similarity was expected to exist between these SAR models and carcinogenesis. Thus SAR models that contain significantly more similar features than expected can be considered as having mechanistic similarities. These relationships can cover several

levels of biological complexity and perhaps ease in the identification of underlying mechanisms. For example, an SAR relationship has been identified between *Salmonella* mutagenicity and carcinogenicity [3]. Arguably, it is easier to understand the mechanistic basis of the induction of mutations in *Salmonella* at the molecular level than the molecular basis of cancer induction. Although based upon analyses of the mutagenic spectrum, one might hypothesize that in carcinogenesis, the molecular basis is similar (*i.e.*, mutation of a suppressor gene (*e.g.* p53)).

However, we devised a more informative method to investigate mechanistic phenomena [4]. A copy of this manuscript is included at the end of this report. Basically, instead of simply analyzing substructure overlap between various toxicological phenomena, we sought to develop a method richer in information that describes these phenomena. Thus, we used the full capabilities of the CASE/MULTICASE system to develop a more information intensive approach to explore mechanistic similarities between biological/toxicological phenomena. It is expected that this new approach will be very successful in analyzing the relationship between estrogenicity and carcinogenicity. This approach has been successfully used in exploring the relationships between inhibition of gap junctional intracellular communication with other toxic phenomena [5].

The remainder of this report will give details for each task described in the statement of work.

Task 1: *Development of criterion for the acceptance and inclusion of experimental data in databases. To obtain this goal, the expertise of Drs. Billy Day and Marjorie Romkes will be utilized.*

Task 2: *Literature Search and collection of data to develop estrogen receptor competitive binding assay database.*

Tasks 1 and 2 proved to be difficult, time consuming and very rewarding. Over 150 published articles were collected that contained data pertaining to the ability of various chemicals to bind to the estrogen receptor (ER). The quantitative measure here is expressed as relative binding to affinity (RBA) to the ER relative to estradiol. Some reports covered one chemical while many included a large series of chemicals. Overall, ER RBAs were gathered from approximately 1400 chemical experiments. Portions of these have been tested multiple times by different researchers. These data will be used to assess the overall consistency of the method. At first pass, the data seem to be consistent.

Originally, one learning set and SAR model was anticipated for ER RBA data. However, researchers have used in excess of seven different test species/systems to measure RBA. These include using ERs from mice, rats, lambs/sheep, calf, human uterus, MCF-7 cells, and clonal assays. It was concluded that multiple models would be made for the endpoints with the most data. These are calf (n=370), rat (n=354) and mouse (n=168). An additional problem encountered was that different researchers used different temperatures to measure RBA (*e.g.*, from 0C to 37C). Where data existed for one chemical being tested at multiple temperatures, there were often large differences

observed between cold and warm conditions. In order to address the potency of these chemicals during QSAR analysis, we derived a regression equation that is capable of adjusting for the temperature variation. All models are adjusted to 0C for consistency.

A small amount of data has been gathered for human ER RBA. Unfortunately, there is not enough to derive an SAR model. However, these data will be able to serve as outside validation sets to see how well the derived SAR models can assess chemical activity in human systems.

Task 3: Literature Search and collection of data to develop estrogen-dependent growth (e.g., MCF-7 cell line) database.

To date, two learning sets and SAR models have been derived for MCF-7 estrogen-dependent growth. The data are all taken from the publications of Dr. Anna Soto. This assures there are no interlaboratory differences. The first model is for relative proliferative potency (RPP) (n=122), which measures the amount of a test chemical needed to maximally stimulate MCF-7 growth. The second model is relative proliferative effect (RPE) (n=122), which measures how much cell growth a chemical can induce compared to estradiol.

Task 4: Literature Search and collection of data to develop mouse uterine weight gain database.

Approximately 20 papers have been collected with data on approximately 100 chemicals for their ability to stimulate uterine weight gain. Although the original task is for mouse only, this is being broadened to include rat data as well. Moreover, this assay is also used to measure antiestrogenicity by measuring if a chemical can inhibit the stimulator effect of estradiol. If sufficient data are available, an SAR model will also be created for antiestrogenicity. It is expected that these models will be derived within two months.

Task 5: Creation of MULTICASE databases associated with tasks 2-4.

SAR models have been created for RBA data (Task 2) and MCF-7 data (Task 3). The SAR model for uterine weight gain is in development.

Task 6: Validate the predictivity of each database in conjunction with MULTICASE.

Validation, as used here, depicts how well the derived models are able to predict the activity (i.e., estrogenicity) of chemicals not contained in the model. These models can therefore be used to assess the activity of environmental chemicals, including phytoestrogens. Of the models derived (Table 1), it can be seen that they are all over 70% predictive. These are very acceptable results and on the order achieved for other toxicological SAR models. The remaining models should take less than one month to validate.

Table 1. Validation results for various estrogenicity SAR models.

Model	Sensitivity	Specificity	Concordance
Calf ER RBA	0.73	0.71	0.72
Rat ER RBA	N/a	N/a	N/a
Mouse ER RBA	N/a	N/a	N/a
MCF-7 RPP	0.72	0.81	0.77
MCF-7 RPE	0.80	0.72	0.76
Uterine weight	N/a	N/a	N/a

Task 7: *MULTICASE structural analyses of each database.*

The databases listed in Table 1 have undergone initial structural analysis. The remainder should be done in less than one month.

Task 8: *Identification of congeneric sets of estrogens that may act through similar mechanisms. Investigate various attributes of chemicals within each set to determine if various structural or physical-chemical properties modulate the activity of these chemicals within each database.*

To be completed.

Task 9: *Identify structural similarities between the three estrogenicity databases to determine whether the databases are, in fact, measuring the same phenomena (i.e., estrogenicity).*

To be completed. This task obviously has been expanded to include the additional SAR models discussed above. Moreover, specific similarities will be assessed for the individual SAR model included under ER RBA, MCF-7, and uterine weight increase.

Task 10: *Identify structural similarities between the estrogenicity database and extend MULTICASE database for carcinogenicity, toxicity and genotoxicity to identify possible similarities in structural determinants and mechanisms of action.*

To be completed. This task has been expanded to include the new method for exploring mechanistic relatedness between various toxicological endpoints described above.

To conclude, no insurmountable obstacles exist and the completion of the work required for this project should be accomplished by the end of summer. Several papers describing the results of this work will be submitted for review shortly thereafter. It is anticipated that this entire project will be completed by December 1999.

References

- (1) Cunningham, A. R., Rosenkranz, H. S., Zhang, Y. P., Klopman, G. (1998). Identification of "genotoxic" and "non-genotoxic" alerts for cancer in mice: The carcinogenic potency database. *Mutat. Res.* **398**, 1-17.
- (2) Cunningham, A. R., Rosenkranz, H. S., Klopman, G. (1998). Identification of "genotoxic" and "non-genotoxic" alerts for cancer in rats: The carcinogenic potency database. *Mutat. Res.* **405**, 9-28.
- (3) Rosenkranz, H. S., Klopman, G. (1990). The structural basis of the mutagenicity of chemicals in *Salmonella typhimurium*: The National Toxicology Program Data Base. *Mutat. Res.* **228**.
- (4) Pollack, N., Cunningham, A. R., Klopman, G., Rosenkranz, H. S. (1999). A new approach for evaluating mechanistic relatedness among toxicological phenomena. *SAR and QSAR in Environmental Research* **in press**.
- (5) Rosenkranz, H. S., Pollack, N., Cunningham, A. R. (submitted). Exploring the relationship between inhibition of gap junctional intracellular communication and other biological phenomena.

Key research accomplishments

- Published two papers describing SAR models for mouse and rat carcinogens
- Developed more advanced method for assess mechanistic similarities between toxicological endpoints
- Derived various models for estrogen receptor binding
- Derived regression model to standardize for temperature variation in ER RBA experiments
- Derived two models for MCF-7 estrogen-responsive growth

Reportable outcomes

Manuscripts, abstract, presentations

- Pollack, N., Cunningham, A.R., Klopman, G., Rosenkranz, H.S. (1999) A new approach for evaluating mechanistic relatedness among toxicological phenomena. SAR and QSAR in Environmental Research. In press
- Rosenkranz, H.S., Cunningham, A.R., Zhang, Y.P., Klopman, G. (1999) Applications of the CASE/MULTICASE SAR to environmental and public health situations. SAR and QSAR in Environmental Research. In press
- Rosenkranz, H.S., Cunningham, A.R., Zhang, Y.P., Claycamp, H.G., Macina, O.T., Sussman, N.B., Grant, S.G., Klopman G. (1999) Development, characterization and application of SAR models. SAR and QSAR in Environmental Research. In press
- Cunningham, A.R., Rosenkranz, H.S., Klopman, G. (1998) Structural analysis of a group of phytoestrogens for the presence of a 2-D geometric descriptor associated with non-genotoxic carcinogens and some estrogens. Proceeding for the Society for Experimental Biology and Medicine 217: 288-292.
- Cunningham, A.R., Rosenkranz, H.S., Klopman, G. (1998) Identification of "genotoxic" and "non-genotoxic" alerts for cancer in rats: The carcinogenic potency database. Mutation Research 405: 9-28.
- Cunningham, A.R., Rosenkranz, H.S., Zhang, Y.P., Klopman, G. (1998) Identification of "genotoxic" and "non-genotoxic" alerts for cancer in mice: The carcinogenic potency database. Mutation Research 398: 1-17.
- Rosenkranz, H.S., Liu, M., Cunningham, A.R, Klopman, G. (1997) Application of structural concepts to evaluate the potential carcinogenicity of natural products. SAR and QSAR in Environmental Research 5: 79-98.
- Cunningham, A.R., Klopman, G., Rosenkranz, H.S. (1997) A dichotomy in the lipophilicity of natural estrogens/xenoestrogens and phytoestrogens, Environmental Health Perspectives Supplements 105(Suppl3): 665-668.

Degrees obtained that are supported by this award

Ph.D., Environmental and Occupational Health, University of Pittsburgh, 1998

Informatics such as databases

Databases to date Mouse estrogen receptor binding affinity
 Rat estrogen receptor binding affinity
 Lamb estrogen receptor binding affinity
 MCF-7 estrogen receptor binding affinity
 Human estrogen receptor binding affinity
 Miscellaneous estrogen receptor binding affinity

Relative proliferative potency estrogenic effect in MCF-7 cell line

Relative proliferative potency estrogenic effect in MCF-7 cell line

Funding applied for based on work supported by this award

Pending

Title: Computational analysis of female carcinogens and the identification of breast cancer agents

Agency: US Army Medical Research and Materiel Command, Breast Cancer Research Project

Title: K02 Career Development Award: Computational modeling of endocrine disruptors

Agency: National Institutes of Health

Title: Computational modeling to investigate whether a link exists between exposure to endocrine disruptors and developmental toxicity

Agency: March of Dimes

Received

Title: Structure-activity relationship modeling of environmental estrogens

Agency: University of Pittsburgh Competitive Medical Research Fund

Employment or research opportunities applied for and/or received based on experience/training supported by this award

Postdoc, Harvard School of Public Health, declined by candidate

Assistant Professor, University of Pittsburgh, accepted